Proteomic Analysis of Post-pollination Senescence in Petunia Flowers

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Abstract

The senescence of vegetative and floral tissues can have a detrimental impact on the quality and subsequent value of agricultural and horticultural crops. To global analyses of protein expression during flower senescence, we are therefore using a proteomic approach to identify components of the senescence program in *Petunia x hybrida* cv Mitchell Diploid flowers. Total soluble proteins were extracted from petunia corollas at 24, 48, and 72 hours after flower opening (i.e. unpollinated, nonsenescing flowers) and at 24, 48, and 72 h after pollination (i.e. senescing flowers). Two- dimensional gel electrophoresis (2DE) was used to identify those proteins that were differentially expressed in nonsenescing (unpollinated) and senescing (pollinated) corollas. PDQuest image analysis (BioRad) software was used to identify those proteins up or down regulated by two fold in pollinated corollas. One hundred forty differentially expression proteins were identified. Most of these were identified by comparing 72 h unpollinated to 72 h pollinated corollas. LC-tandem mass spectrometry (LC-tandem MS) was used to determine the identity of these proteins. Searching the NCBI nonredundant protein and petunia translated EST database we have been able to assign a putative identification to greater than 90% of these proteins. Identified proteins are involved in many metabolic pathways including proteolysis; nuclei acid, cell wall and lipid catabolism; and signal transduction. To further characterize the role of these proteins in flower senescence, we will knockdown the expression of the corresponding genes using virus-induced gene silencing.

Results

Figure 1. Pollination accelerates petunia flower senescence. Petal wilting is apparent at 48 h after pollination. U, unpollinated; P, pollinated.

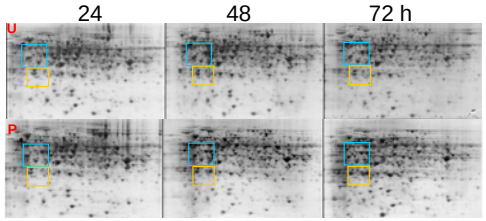

Figure 2. Expression profiling of corolla proteins during pollination- induced petunia flower senescence using 2-DE. Representative gels from the 3 replicates comparing protein profiles of corollas from unpollinated (U) flowers and pollinated (P) flowers at 24, 48, and 72 h after pollination. Areas in the blue and yellow boxes are amplified to identify specific protein differences between pollinated and unpollinated corollas in Fig 4.

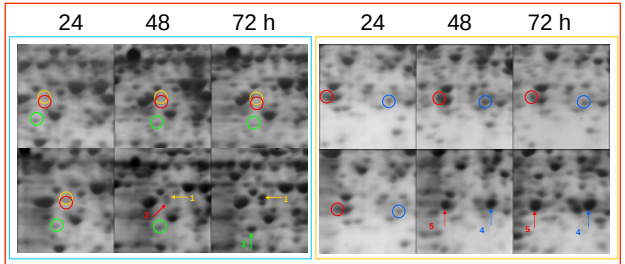

Figure 4. Five proteins that are upregulated in petunia corollas at 48 and/or 72 hours after pollination. These proteins were identified using capillary column LC-tandem MS and their putative identity is described in table 2.

Table 2. Identities of putative proteins from Figure 4

Putative Identity	Accession No.	# Peptide (Exact/Homologous) Match	Sequence Coverage (%)
#1 Endonuclease	50657596	8 (4/4)	32
#2 Adenosine kinase isoforms 1S	51949800	3 (2/1)	13
#3 20S proteasome alpha 6 subunit	22947842	9 (7/2)	36
#4 Cysteine proteinase PhCP10	52546926	7 (7/0)	42
#5 Cysteine proteinase P21	945081	8 (7/1)	23

Introduction

Senescence represents the last stage of flower development leading to death. The programmed death of flower petals is an active process that is executed via a defined genetic program (Jones, 2004). Pollination accelerates petal senescence and allows the plant to break down macromolecules and organelles and remobilize nutrients to developing tissues (Langston et al., 2005; Jones et al., 2005). Petal senescence is accompanied by changes in the activity of specific enzymes and the abundance of certain proteins (Jones, 2004). Treating flowers with cycloheximide, an inhibitor of protein synthesis, delays flower senescence (Rubinstein, 2000). These experiments confirm the importance of de novo protein synthesis in the regulation of flower senescence. To further understand the regulation of flower senescence we employed a proteomic approach, utilizing 2-dimensional gel electrophoresis (2-DE) and mass spectrometry to identify proteins that were differentially expressed in senescing and nonsenescing petals.

Materials and Methods

Petunia x hybrida cv Mitchell Diploid petals (collectively called the corolla) were collected from unpollinated and pollinated flowers at 24, 48, and 72 h after flower opening. Total proteins were extracted using the methods described in Coaker et al., 2004. Two hundred micrograms of total protein was used to rehydrate 11-cm immobilized pH gradient (IPG) strips (pI 5-8; BioRad), and isoelectric focusing using the Protean IEF Cell (BioRad) was run overnight. Equilibrated strips were then subjected to SDS-PAGE (12.5%) for second dimension separation. At least three replicate gels (3 biological replications of 8 corollas each) from each time point were evaluated. Gels were stained with Gel code blue (Pierce) and gel images were analyzed with PDQuest v. 7.40 (BioRad). Proteins that were differentially expressed between pollinated and unpollinated corollas were excised from the SDS-PAGE gels and digested with trypsin. The digested peptides were analyzed using capillary column LC-tandem MS (Finnigan LCQ ion trap mass spectrometer, Cleveland Clinic Proteomics Laboratory) acquiring full scan mass spectra to determine peptide molecular weights and product ion spectra to determine amino acid sequences in successive instrument scans. Each of the datasets was searched against both the NCBI non-redundant protein database using the Mascot search program and the OSU Solanaceae protein database using the Sequest search program.

Differentially Expressed Proteins	Time points and Treatments					
	24 U vs 24 P		48 U vs 48 P		72 U vs 72 P	
	No.	% (total)	No.	% (total)	No.	% (total)
Upregulated proteins	0	0	74	12.8	113	20
Down regulated proteins	0	0	41	7.1	57	10.1
Total	0	0	115	19.9	170	30.1

Table 1. Profiling of differentially expressed proteins during petunia flower senescence. No significant protein differences were detected between 24 h pollinated (P) and 24 h unpollinated (U) corollas. Differentially expressed proteins were detected between 48 P and 48 U and 72 P and 72 U corollas. Proteins were defined as upregulated if they were not detected in unpollinated corollas and were detected in pollinated corollas or if they increased in abundance in pollinated corollas. Proteins were defined as down regulated if they were detected in unpollinated but not in pollinated corollas or if they decreased in abundance in pollinated corollas. Significant differences among treatments were detected by the *t*-test (P≤0.05).

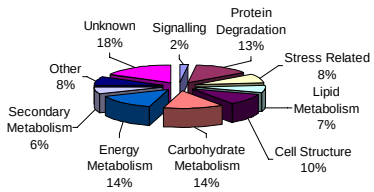

Figure 3. Function classification of upregulated proteins. Eighty seven upregulated protein spots were sequenced. We have assigned a putative identification to >90% of these proteins based on searching the NCBI non-redundant protein database and the OSU Solanaceae protein database. As protein synthesis is required during senescence, we chose to focus on the upregulated proteins.

Discussion

The majority of the proteins up regulated following pollination and during corolla senescence were those involved in the catabolism of macromolecules. It is well known that protein and nucleic acid degradation is a large component of the petal senescence program in petunias. The Jones' lab has previously shown that cysteine protease activity increases during corolla senescence and that the gene encoding the cysteine protease PhCP10 increases during corolla senescence (Jones et al., 2005). Similarly activity of a senescence-specific endonuclease has been shown to correlate with DNA fragmentation in senescing petunia corollas (Langston et al., 2005). At this time the role of the other proteins, including the Adenosine kinase isoforms 1S, in the senescence program is unclear.

Conclusions

- Differential protein expression can be detected during senescence at a large scale using 2-DE.
- Using MS spectra to search the NCBI and OSU Solanaceae databases we have assigned a putative identity to over 90% of the petunia proteins.
- The protein classifications indicate that senescence-related proteins are involved in multiple signaling and metabolic pathways.
- To further characterize the role of these proteins in flower senescence we will knockdown the expression of the corresponding genes using virus-induced gene silencing.

References

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